EXHIBIT A

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Reduced intensity conditioning regimens

A novel reduced intensity regimen for allogeneic hematopoietic stem cell transplantation associated with a reduced incidence of graft-versus-host disease

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Summary:

In all, 55 patients at high risk or ineligible for a conventional allogeneic hematopoietic stem cell transplant (HSCT) received a regimen consisting of extracorporeal photopheresis, pentostatin, and reduced dose total body irradiation. The median age was 49 years (18–70 years); 44 received a sibling and 11 an unrelated HSCT; 44% were over the age of 50 years and 31% had undergone a prior HSCT. Graft-versus-host disease (GVHD) prophylaxis consisted of cyclosporine and methotrexate. Full donor chimerism was documented in 98% by day +100. The 1000-day nonrelapse mortality was 11%. The median follow-up is 502 days (154–1104 days). The 1- and 2-year overall survival (OS) and event-free survival (EFS) are 67, 58 and 55%, and 47%, respectively. Patients who had not received a prior HSCT or had less than three prior chemotherapy regimens had a 71% OS and 67% EFS at 1 year. Greater than grade II aGVHD developed in 9% and chronic GVHD (cGVHD) in 43%, and extensive in 12% and limited in 31%. Of the patients, 86% who engrafted had a disease response, 72% had complete and 14% partial responses. This novel reduced intensity preparative regimen was well tolerated and associated with a low incidence of transplant-related mortality and serious acute and cGVHD.

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Current allogeneic hematopoietic stem cell transplant (HSCT) preparative regimens are designed to administer the maximally tolerated doses of chemotherapy and/or radiation therapy in an attempt to eradicate residual disease, ablate host hematopoiesis, and immune suppress the recipient to ensure engraftment of donor hematopoietic stem cells (HSC). While this approach is curative for many patients, it is also associated with 30–50% transplant-related mortality (TRM) in patients over the age of 50 years.[1] While advances in supportive care and the management of acute graft-versus-host disease (aGVHD) have decreased the TRM and morbidity associated with an allogeneic HSCT, its application remains limited to patients who are able to tolerate the side effects of high-dose chemotherapy administered as part of a conventional preparative regimen.[2]

Recent studies suggest that disease control and engraftment of allogeneic HSCT can be achieved following nonablative doses of chemoradiotherapy.[3–5] Moreover, in various diseases, the beneficial effects of the allogeneic HSCT result from an immune-mediated graft-versus-malignancy (GVM) effect and not from the high doses of chemotherapy administered in the preparative regimen.[5–8] Stable full or partial donor stem cell engraftment following the administration of a nonablative, reduced intensity preparative (RIT) regimen may also provide a platform for subsequent immune modulation to augment the GVM effect.[7–9] Compared to conventional, ablative preparative regimens, RIT regimens are generally associated with a decrease in TRM and other transplant-related complications.[3,7,10,11] However, despite a decrease in regimen-related toxicities, serious aGVHD and cGVHD remain a major cause of TRM and morbidity in high-risk and elderly patients.[7,10,11,12] GVHD and its treatment is associated with a delay in immune reconstitution and an increase in infectious and late transplant-related complications.[7,11,13–16] Heavily pretreated or older patients with comorbid conditions, and those who have received a prior autologous HSCT are at a high risk of developing transplant-related complications and aGVHD.[17–19] T-cell depletion strategies have been successful in abrogating aGVHD, but result in a delay in immune reconstitution, the development of mixed chimerism, and an increased incidence of late infectious complications.[14,20,21]

We report on a novel RIT conditioning regimen incorporating extracorporeal photopheresis (ECP), pentostatin, and low-dose total body irradiation (TBI) to

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modulate both host and donor effector cells.[22–25] Our results in 55 high-risk patients with various malignancies demonstrate that this regimen was associated with the early establishment of full donor chimerism, durable clinical reponses, without the need for subsequent donor lymphocyte infusions (DLI), and a low incidence of both TRM and GVHD.

Materials and methods

Patients

In all, 55 consecutive high-risk patients with various malignant disorders received an RIT allogeneic HSCT transplant. Patients were eligible for the treatment if they had a disease for which an allogeneic-matched sibling or matched unrelated donor HSCT is a generally accepted therapy. All patients entered on study were not eligible for or at high risk for TRM associated with conventional, ablative, and allogeneic HSCT. Patients were determined to be at high risk because of one or more of the following criteria: (1) age, greater than 50 or 55 years for chronic myelogenous leukemia (CML); (2) comorbid diseases or disorders that were felt to increase substantially the risk of an allogeneic HSCT; or (3) extensive prior treatments that would increase the toxicity of a conventional allogeneic transplant preparative regimen, including patients who had received a prior conventional allogeneic or autologous HSCT.

Patients received an allogeneic HSCT from a related donor ($n=44$) or a matched unrelated donor ($n=11$). The HSC source was bone marrow in 53 and peripheral blood stem cells (PBSCs) in two. All the patients and donors were HIV antibody negative. All patients had an ECOG performance status of less than 3, a creatinine clearance of greater than 25 ml/min, and a total bilirubin of less than two times normal prior to starting the preparative regimen. All patients signed an IRB-approved informed consent. Unrelated donors were identified and consented through the National Marrow Donor Program according to the accepted guidelines.

The patient characteristics are listed in Table 1. The median age of the patients at the time of the transplant was 49 years (range 18–70 years). All patients with myelodysplastic syndrome (MDS) had an IPSS score of >1 and were either red cell or platelet transfusion dependent. Patients with chronic lymphocytic leukemia and cutaneous T-cell leukemia were refractory to greater than 2 conventional treatment regimens. All patients with multiple myeloma relapsed after receiving at least two treatment regimens. The patients with myelofibrosis and myeloid metaplasia (MMM) had advanced disease and were red cell transfusion dependent with massive splenomegaly and hepatomegaly and underwent a splenectomy prior to starting the preparative regimen. All renal cell carcinoma (RCC) patients had a prior nephrectomy with documented metastatic disease unresponsive to recombinant IL-2. In all, 17 patients (31%) had received a prior HSCT (15 autologous, two conventional allogeneic). The median time from the first HSCT to the RIT allogeneic HSCT was 366 days (range 136–3639 days). In all, 10 patients (18%) had received at least three prior treatment regimens before starting the preparative regimen.

Table 1 Patient characteristics, $N=55$

Median age	*49 years (range 18–70 years)*
Median time: diagnosis to transplant	*366 days (range 136–3639 days)*
	N (%)
<50 years	31 (56)
⩾50 years	24 (44)
Gender	
Male	29 (53)
Female	26 (47)
Diagnosis	
AML	11 (20)
MDS	14 (25)
NHL/HD	12 (22)
CML	8 (15)
MMM	2 (4)
Multiple myeloma	2 (4)
Renal cell carcinoma	3 (5)
CLL/CTCL	3 (5)
Prior therapy	
Autologous BMT	15 (27)
Allogeneic BMT	2 (4)
Chemotherapy	
3 courses	10 (18)
Biologic therapy	4 (7)
No prior therapy	18 (33)
Relapse/refractory disease	33 (60)

AML = acute myelogenous leukemia; MDS = myelodysplatic syndrome; MMM = myelofibrosis and myeloid metaplasia; CML = chronic myelogenous leukemia; NHL = non-Hodgkin's lymphoma; HL = Hodgkin's lymphoma; CLL = chronic lymphocytic leukemia; CTCL = cutaneous T-Cell leukemia.

In all, 24 (44%) patients were over the age of 50 years. Concurrent medical problems that precluded a conventional allogeneic HSCT were present in 39% and included chronic active hepatitis B ($n=1$), previous extensive pulmonary aspergillosis ($n=2$), morbid obesity ($n=3$), and symptomatic coronary artery disease, or cardiopulmonary disease ($n=15$). In total, 33 patients (60%) had relapsed or had progressive disease (PD) at the time of the allogeneic HSCT.

The transplant-related characteristics are outlined in Table 2. All donors and recipients had molecular typing for HLA-A, -B, DRB1, and DQB1.

In the analysis of overall survival (OS) and event-free survival (EFS), patients were grouped into two categories based on previously described risk factors.

Group I ($n=27$: 49%), the highest risk, included patients who had received a prior allogeneic or autologous HSCT or had received >3 treatment regimens prior to the allogeneic HSCT; group II ($n=28$: 51%) included all other patients with standard high-risk features as described previously.[10,11] The two groups were otherwise similar with regard to age, time from diagnosis to the HSCT, baseline liver and renal function, performance status,

Table 2 Transplant characteristics, $N = 55$

Type of transplant	*N*	%
Related, HLA match	38	69
Related, 5/6 HLA match	6	11
Unrelated, HLA match	11	20
CMV status (recipient/donor)		
+/+	14	25
+/−	8	15
−/+	7	13
−/−	26	47
Donor–recipient gender		
Male > male	19	35
Male > female	14	25
Female > male	10	18
Female > female	12	22

and disease characteristics at the time of the RIT allogeneic HSCT.

Preparative regimen

The preparative regimen consisted of ECP administered on 2 consecutive days followed by 48 h of continuous infusion (CI) pentostatin, and then 600 cGy TBI delivered in three 200 cGy fractions (Figure 1). Day 0 was the day of the HSCT infusion. ECP was administered on days −7 and −6 as an outpatient, with the XTS Photopheresis System (Therakos, Exton, PA, USA) by the standard methods as described previously.[26]

Pentostatin was administered at a dose of 4 mg/m^2/day by a continuous intravenous infusion for 2 consecutive days (days −5 and −4). The pentostatin dose was reduced by 50% for a CrCl < 50 ml/min or a serum creatinine > 2 mg/dl.

TBI was given in three 200 cGy fractions on days −3 and −2. Two patients who previously received 1200 cGy, TBI for a prior allogeneic transplant, were treated with a total dose of only 400 cGy delivered in two 200 cGy fractions. TBI was delivered with parallel-opposed lateral 24 MV photon beams. Patients with CML received 500 cGy splenic radiation administered in 100 cGy fractions for 5 days prior to the initiation of photopheresis using parallel-opposed AP/PA portals on a megavoltage linear accelerator with energies of 4 or 6 MV as described previously.[27] Non-stimulated donor bone marrow was collected on day 0 by the usual procedure and infused the same day. PBSCs were mobilized with subcutaneous granulocyte colony-stimulating factor at 10 μg/kg for 4 consecutive days. PBSC collection was performed by leukapheresis on day 4 as described previously.[28] All stem cells were infused through a central venous catheter on day 0.

Chimerism

Chimerism studies were performed at baseline, at engraftment, and on days 30, 60, 100, and 1 year after the transplant. In sex-mismatched transplants, fluorescent *in situ* hybridization studies for X or Y chromosomes were used to determine chimerism on the same schedule. Chimerism in all sex-matched transplants was assessed by restriction fragment length polymorphisms on peripheral blood T cells, using the GenePrint® SilverSTR™ III Triplex System (Promega, Madison, WI, USA).[29] Full donor chimerism was defined as greater than 95% donor T cells on day 100.

GVHD prophylaxis and supportive care

All patients received GVHD prophylaxis with cyclosporin A (CSA) and two doses of methotrexate (MTX). CSA was administered by CI through an indwelling catheter starting on day −1 and continuing to +50 as described previously.[30] Briefly, CSA was started at a dose of 2.5 mg/kg/day and subsequently adjusted to maintain a whole blood level between 475 and 525 ng/ml, as determined by fluorescence polarization immunoassay (Abbott TDX, Abbott Park, IL, USA).[30] MTX was administered as an intravenous bolus infusion on day +1 (15 mg/m^2) and day +3 (10 mg/m^2). On day +50, patients were switched to oral CSA, (Neoral) at a dose of 5 mg/kg twice. CSA was tapered by 50 mg/day every 2 weeks until reaching 100 mg twice a day, and then oral mycophenolate mofetil (MMF) at an initial dose of 500 mg twice a day was added. MMF was increased to a maximum of 1000 mg twice a day, as tolerated, and CSA was tapered and stopped. MMF was continued until 1 year after the HSCT and then rapidly tapered. The patients with metastatic RCC were started on CI CSA at the same initial dosing schedule, but on day 20 were switched to oral CSA and tapered at a rate of 50 mg/week unless aGVHD developed.

Preparative Regimen	
Day −7, −6	Extracorporeal photopheresis
Day −5, −4	Pentostatin 4mg/m^2/day × 2 days by continuous infusion
Day −3, −2	TBI 200 cGy ×3 (600 cGy total)
Day −1	Rest
Day 0	Hematopoietic Stem Cell Infusion

Figure 1 Preparative regimen.

aGVHD and cGVHD were graded according to standard criteria.[16,31] aGVHD was graded weekly until day +50 and then as clinically indicated. The maximum grade of the aGVHD was used in the analysis. Complete and partial disease responses were assessed using standard disease-specific criteria. Responses were assessed at day 100, 180, and 1 year or as clinically indicated. A cytogenetic complete remission was defined as the absence of the cytogenetic abnormality that defined the disease at one 1 year following the transplant or as clinically indicated. In patients with leukemia, MDS or MMM progression was defined by standard clinical, morphologic, and cytogenetic criteria.

Antibacterial and antiviral prophylaxes were performed according to institutional protocols. Ciprofloxacin (500 mg p.o. q12h) and acyclovir (200 mg p.o. q.i.d.) were administered starting on day +1 and continued until neutrophil engraftment. All patients received intravenous immunoglobulin (0.5 g/kg) weekly for four doses following engraftment, then every other week for four doses and then monthly for four doses, which was the standard institutional practice at the initiation of the trial for patients undergoing an allogeneic stem cell transplant. All patients were housed in high-efficiency particulate air (HEPA) filtered rooms. Trimethoprim-sulfamethoxazole prophylaxis for *Pneumocystis carinii* infection (PCP) was started following engraftment and continued for 1 year. Patients requiring treatment for cGVHD continued to receive PCP prophylaxis. Patients were assessed for cytomegalovirus (CMV) reactivation using a polymerase chain reaction-based (PCR) assay.[32] CMV reactivation was assessed weekly following engraftment until day +100 and then as clinically indicated. Patients with reactivation of CMV were treated with intravenous or oral ganciclovir according to institutional protocols.

Regimen-related toxicities were graded and recorded according to the Bearman criteria.[33] Maximum toxicity grades are shown in Table 3.

Clinical end points and statistical analysis

OS and EFS were calculated from day 0, the day of the transplant. OS analysis included all deaths regardless of the cause. EFS included relapse, progression, or death from any cause. Relapse was defined as recurrence of the underlying disease for patients in complete remission; progression was defined by standard disease-specific criteria for patients with disease at the time of the allogeneic HSCT. Nonrelapse-related mortality (NRM) was defined as death that was not related to disease relapse or progression. Neutrophil engraftment was defined as an absolute neutrophil count of greater than 0.5×10^9/l for 3 consecutive days. Platelet engraftment was defined as a platelet count of greater than 20×10^9/l independent of platelet support for greater than 3 days.

All actuarial survival estimates were calculated using the Kaplan–Meier method with JMP statistical software (SAS Institute Inc., Cary, NC, USA).[34] Disease-specific responses were evaluated on days 100, 180, 360, or as clinically indicated. Comparisons between groups for OS and EFS were performed by the log-rank test. The association of various patient characteristics with outcomes was examined using the Wilcoxon's rank-sum test for continuous characteristics. The analysis was based on a mean follow-up of 503 days. All patients are at least 100 days following the HSCT.

Table 3 Bearman grade III or IV toxicity, $N = 55$

	CNS	GI	Mucositis	Hepatic	Lung	Renal	Bladder	Heart
N	2	2	0	5	2	4	0	1

Results

Regimen-related toxicities and adverse events

Regimen-related toxicities occurring in the first 100 days after the transplant are summarized in Tables 3 and 4. None of the patients developed painful mucositis, severe nausea or vomiting, veno-occlusive disease (VOD) of the liver, or hemorrhagic cystitis. All patients developed alopecia secondary to the TBI. In 14 patients (25%), the serum creatinine rose to twice the baseline or greater and was related to the high targeted serum level of CSA or pre-existing kidney disease. The mean peak creatinine was 2.4 mg/dl ± 0.20 (s.e.m.). Two patients required dialysis; one patient developed renal failure in the context of multiorgan failure following an unrelated donor transplant and one patient with recurrent multiple myeloma had a history of renal dysfunction and a pretreatment CrCl < 50 ml/min. In the remaining patients, renal dysfunction resolved with standard supportive care. Transient facial erythema occurred during pentostatin infusion in 37% of patients and resolved without treatment. Three patients developed transient CSA-associated central nervous system toxicity, including one with a seizure and two with confusion and fatigue. Four patients developed a bilirubin of greater than 10 mg/dl, including two who died of multiorgan failure following an unrelated donor transplant. The mean peak total bilirubin was 4.36 ± 0.79 mg/dl (s.e.m.). The elevated bilirubin levels were attributed to either CSA, concomitant medications, GVHD, or hemolysis. MMF was adjusted for the neutrophil and platelet count. There were no untoward reactions to the prolonged course of MMF. CMV reactivation occurred in 10 patients (18%), including eight of the 22 (36%) CMV seropositive patients. Two CMV seronegative patients with seropositive

Table 4 Causes of death

Time	GVHD	Infections	Relapse/PD	Other[a]	Death (%)	NRM (%)
<100 days	1	2	3	3	16	11
>100 days	3	1	8	1	24	9

PD = progressive disease; NRM = nonrelapse mortality.
Cardiopulmonary ($N = 3$).
[a]Other = CNS bleed ($N = 1$).

donors developed a positive PCR assay for CMV. Only one patient developed clinical evidence of CMV disease.

The incidence of renal insufficiency was significantly different between the two risk groups. The mean creatinine for patients in the very high-risk, group I, and standard risk group, group II, were 2.2 ± 0.20 (s.e.m.) and 2.8 ± 0.35 (s.e.m.), respectively ($P = 0.05$). There was no difference between the two groups with regard to maximum bilirubin or the incidence of CMV reactivation.

Nine patients developed grade III–IV Bearman toxicities (Table 3). Nine patients (16%) died prior to day 100; causes of death included PD ($n = 3$), cardiopulmonary complications including a patient with previous anthracycline-associated cardiomyopathy ($n = 2$), infection/multiorgan failure ($n = 2$), and intracranial bleed ($n = 1$) in a patient with extensive cardiovascular disease. One patient did not comply with the prescribed antibacterial or GVHD prophylaxis and died of complications from aGVHD on day 90. Five of the nine deaths (56%) occurred in patients in the high-risk group, and seven of the nine patients who died (78%) had residual disease at the time of the transplant. The NRM rate in the first 100 days was 11%. Five patients died after day +100, including three patients with GVHD. Eight patients relapsed after day +100. The 1-year nonrelapse mortality was 23%.

Engraftment and chimerism studies

Full donor chimerism was documented in 50 of the 51 evaluable patients (98%). Four patients (7%), all with acute myelogenous leukemia (AML) in relapse, progressed following the HSCT and did not engraft with donor HSC at the time of death or day 100. One patient died on day +36 from infectious complications and did not show evidence of donor engraftment on day +30. No patient required DLI to maintain engraftment. All patients in remission demonstrated full donor chimerism at the 1-year evaluation. The median time to an ANC $> 500/\mu l$ was 17 days (range 9–39 days) and to platelets $> 20\,000/\mu l$ was 20 days (range 0–100 days). In two patients, the platelet count did not fall below $20\,000/\mu l$.

Graft-versus-*host disease*

Five (9%) patients developed grade II–IV aGVHD, including two patients (4%) with grade III–IV aGVHD. Both these patients died of multiorgan failure and/or infections as a result of the GVHD. One patient with CML did not comply with the post transplant GVHD or antibiotic prophylaxis and developed grade III aGVHD. There was no difference in the frequency or severity of aGVHD or cGVHD between patients in either risk group I or II. cGVHD developed in 43%, 12% with extensive and 31% with limited. Two patients with extensive cGVHD died from complications relating to the GVHD. None of the patients received prophylactic steroids for GVHD prevention. Only patients with documented GVHD were maintained on a prolonged course of steroids.

Survival and disease response

Times and causes of death are detailed in Table 4. The overall documented disease response was 86%, with 72% complete (CR) and 14% partial responses (PR) (Table 5). In the 44 patients with residual or PD at the time of starting the preparative regimen, the CR rate was 78%. In the 24 patients with AML and MDS, 71% achieved a CR following the RIT HSCT. Four patients with refractory leukemia died of PD. One patient with AML without evidence of leukemia following the HSCT did not meet the criteria of a complete remission and died of complications of the transplant. Seven of the eight patients (87.5%) with CML are alive in hematologic and cytogenetic remission with a median follow-up of 339 days (range 151–1002 days). One patient with CML died on day +90 from complications of GVHD after abruptly discontinuing GVHD and antibiotic prophylaxis. Two patients with MMM are alive at 218 and 604 days post transplant. One patient has had a CR with normal trilineage hematopoiesis and a reversal of marrow fibrosis. The other patient continues to respond with decreasing marrow fibrosis. In both patients, massive hepatomegaly (>16 cm below right costal margin) resolved by day +180 following the HSCT. One of the three patients with RCC is alive at day 202 with radiologically stable disease. The two patients with pulmonary aspergillosis received antifungal therapy following the transplant and in both cases the fungal disease resolved. The patient with chronic hepatitis B received antiviral therapy for 6 months and remains in complete remission without evidence of hepatitis B infection.

The actuarial 1- and 2-year OS for all patients is 67 and 55%, respectively (95% CI 58–82 and 39–71% for 1- and 2-year survival) (Figure 2). At a median follow-up of 502 days (range 154–1104 days), 62% of the patients are alive. The overall EFS is 58 and 47% at 1 and 2 years, respectively (95% CI 50–74 and 31–63%) (Figure 3).

Analysis of prognostic factors revealed that neither the sex of the patient, donor–recipient sex mismatch, age at transplant (≥ 50 years *vs* < 50 years), CMV status (positive *vs* negative), type of transplant (sibling *vs* matched unrelated) were predictive for OS or EFS. The time from diagnosis to transplant (greater or less than 15 months) was predictive for OS, 83 *vs* 44%, ($P = 0.03$). A 1-year survival was not significantly different between patients receiving a sibling or unrelated HSCT (69 *vs* 55%).

Table 5 Responses following the allogeneic HSCT

Diagnosis	*CR (%)*	*PR (%)*	*NR/PD (%)*	*NA (%)*
AML	68		16	16
NHL, CLL, HD	60	20	13	7
CML	88			12
MMM	50	50		
Myeloma	100			
RCC		33	33	33

CR = Complete remission; PR = partial remission; NR = no response; PD = progressive disease; NA = not evaluable; AML = acute myelogenous leukemia; MDS = myelodysplastic syndrome; NHL = non-Hodgkin's lymphoma; CLL = chronic lymphocytic leukemia; HD = Hodgkin's disease; CML = chronic myelogenous leukemia; MMM = myelofibrosis and myeloid metaplasia; RCC = renal cell carcinoma.

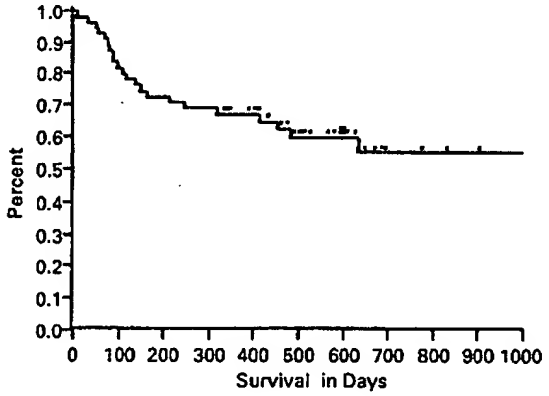

Figure 2 Kaplan–Meier curve showing the survival in days. Day 0 was the day of the HSCT.

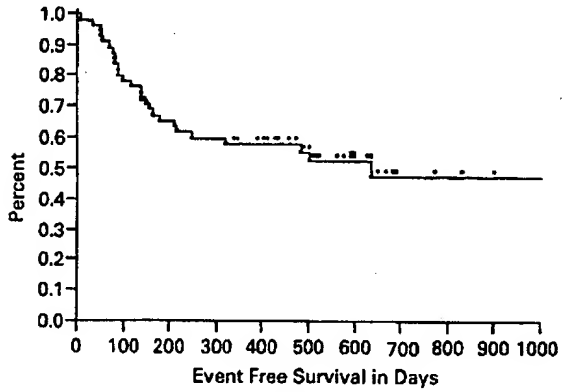

Figure 3 Kaplan–Meier curve showing EFS. Day 0 was the day of the HSCT.

OS and EFS were significantly different in the two risk groups. The 1-year OS was 62% for group I (high risk) *vs* 71% for group II (standard risk) ($P=0.05$ log rank). A 1-year EFS was 52% for group I *vs* 64% for patients in group II, ($P=0.04$ log rank) (Figure 4a and b).

In the 33 patients without prior allogeneic HSCT, and less than three prior chemotherapy regimens who received a matched sibling donor graft, there were no nonrelapse-related deaths in the first 100 days. In these 33 patients, the 1-year OS and EFS were 85 and 72%, respectively. (Figure 5)

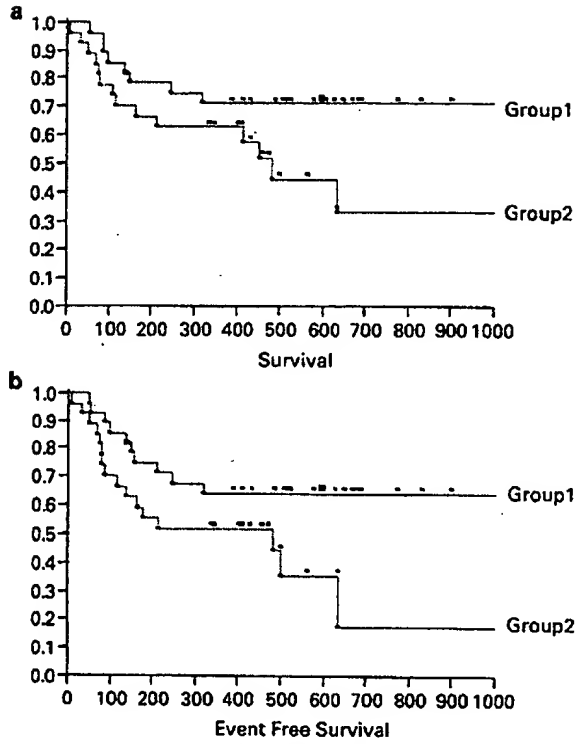

Figure 4 Kaplan–Meier curves showing OS and EFS in days according to risk group. (a) Group I, high-risk group *vs* group II, standard risk group. High-risk group included patients who received >3 prior treatments or a prior transplant ($P=0.05$). (b) EFS ($P=0.04$).

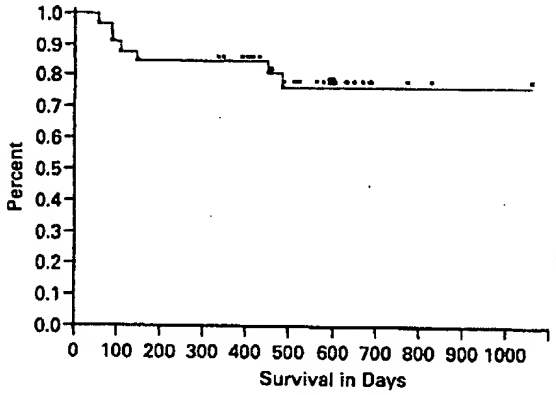

Figure 5 Kaplan–Meier curve showing the survival in days for the 33 patients who received a matched sibling donor transplant and received <3 prior chemotherapy regimens. Day 0 was the day of the HSCT.

Discussion

RIT preparative regimens differ greatly in the intensity of the preparative regimen, the types of the immune suppression utilized to prevent graft rejection, and the intensity and duration of the prophylaxis used to prevent serious aGVHD.[3,5,7,13,35,36] Many regimens result in mixed hematopoietic chimerism, requiring subsequent DLIs to establish full donor chimerism, maintain engraftment, and augment the GVM effect.[7,37–40] Despite decreased regimen-related toxicities and NRM with these regimens, the incidence of serious grade II–IV aGVHD, in non-T-cell-depleted HSCTs, remains at 25–50%. Moreover, RIT allogeneic HSCTs have not resulted in a reduction in the incidence of extensive cGVHD.[12,38–41]

The optimal preparative regimen should insure a high rate of donor HSC engraftment, adequate disease control

in patients with active disease, and preserve the GVM effect with a low incidence of serious aGVHD and cGVHD. Current studies suggest that similar subsets of immune regulatory T cells may be responsible for both HVG and GVH reactions.[20,23,25] Therefore, we postulated that agents that are potentially effective in the treatment of GVHD may also be effective in preventing the graft rejection.[23,42]

In this study, we demonstrated that a preparative regimen consisting of ECP, CI pentostatin, and low-dose TBI was well tolerated and associated with a low NRM, the rapid establishment of full donor chimerism, and a low incidence of aGVHD and cGVHD. Our population of patients was heavily pretreated: 31% had received a prior autologous or allogeneic HSCT, 22% had received greater than three chemotherapy regimens, and 60% had refractory or relapsed disease. The 100-day NRM of 11% reported here compares favorably with other reduced intensity regimens.[5,9–11,36] Moreover, the fractionated 600 cGy TBI and pentostatin were effective in the establishment of early full donor chimerism and controlled active disease in the majority of patients. All evaluable patients demonstrated full donor chimerism and none required the subsequent infusion of donor lymphocytes to maintain engraftment or augment the GVM response. In contrast to other RIT cytoreductive regimens, no patient in our study developed VOD, mucositis, or serious gastrointestinal side effects.[5,10,21,36]

Our regimen was associated with a lower than expected incidence of serious aGVHD and cGVHD. In other RIT regimens using purine analogs and low doses of TBI or melphalan, the incidence of aGVHD ranged from 25 to 50%, compared to the 9% incidence of grade III–IV aGVHD in our study.[5,7,9,11,43] The incidence of aGVHD and cGVHD in the present study is similar to reported preparative regimens that utilized *in vivo* T-cell depletion techniques.[21,40,43] However, in contrast to the T-cell-modified transplants, we did not observe a high incidence of infectious complications, mixed chimerism, or disease relapse.[21,40,43,44] Moreover, the patients in our study were at high risk for the development of aGVHD: 31% received either an unrelated donor or mismatched related donor transplant, 43% were recipients of a sex-mismatched transplant, 44% were over the age of 50 years, and 66% were heavily pretreated or had refractory disease (Table 2).[41]

It is unclear as to which components of our preparative regimen contributed to the low incidence of aGVHD. The majority of the patients received bone marrow as the stem cell source, in contrast to other RIT studies using PBSC. However, recent studies have not demonstrated a significant difference in the incidence of aGVHD based on the source of the HSC.[45] The prolonged course of immune suppression with CSA followed by MMF was administered because the patients were at high risk for developing GVHD. All patients received the same prolonged GVHD prophylaxis, but it is unlikely that the use of two doses of MTX and CI CSA or subsequent course MMF explained the decreased incidence of serious aGVHD or cGVHD.[46]

We administered ECP and pentostatin because both agents have been used in the treatment of patients with refractory GVHD. Prior studies of ECP in patients with aGVHD and cGVHD demonstrated that it was effective in treating both the cutaneous and visceral manifestations of GVHD.[26,47–49] The mechanism of action of ECP in GVHD may involve modulation of T cells and dendritic cells (DCs), the effector cells implicated in ongoing alloreactivity.[26,43,49,50] The effects of ultraviolet A (UVA) and methoxypsoralen on antigen-presenting cells have been demonstrated in murine skin xenograft models in which UVA treatment was associated with a decrease in the number and function of epidermal Langerhans cells.[51] In prior studies of ECP in patients with cGHVD, we demonstrated that ECP inhibited the capacity of circulating DCs to stimulate the proliferation of allogeneic or autologous antigen-stimulated T cells.[26,49] In this regimen, we hypothesized that ECP modulated host DC function and may have contributed, in part, to the attenuation in aGVHD that we observed. The role of host antigen-presenting cells in the initiation of GVHD was demonstrated in a murine transplant model, in which modified host DC failed to recognize Class I antigens on allogeneic T cells.[52] These studies suggested that host-derived DCs have a pivotal role in the initiation of alloreactivity via recognition of minor antigens on infused donor lymphocytes in the context of MHC Class I antigens. Therefore, therapies targeting host DC function that leave donor DCs intact may attenuate aGVHD without impairing immune reconstitution.[52] ECP, pentostatin, or the combination may deplete or modulate host DC function and may have contributed to the low incidence of severe aGVHD observed. A similar mechanism has been proposed to explain the decreased incidence and severity of aGVHD reported in patients who received Campath-1G as part of a preparative regimen.[35]

The use of infusional pentostatin in this regimen was to induce immune suppression and therefore facilitate engraftment.[53] Pentostatin is a potent inhibitor of the enzyme adenosine deaminase, and its mechanism of cytotoxicity differs from the other purine analogs. Pentostatin induces prolonged T-cell depletion and in experimental models has been shown to prevent or decrease the severity of aGVHD when administered prior to the infusion of allogeneic HSCs.[54,55] In a murine allogeneic transplant model, mice receiving a regimen containing pentostatin showed a decrease in the clinical and histologic signs of aGVHD and cGVHD.[54] While pentostatin induces a prolonged depletion of circulating T cells, the long-term effects on host immunoregulatory T cells are not known. Recent pilot studies have also demonstrated that pentostatin is effective in the treatment of refractory aGVHD GVHD.[56,57]

The use of CI pentostatin in this regimen was well tolerated, with minimal mucositis, gastrointestinal side effects, or evidence of tissue damage that may have had a impact on the development of serious aGVHD.[53,55] Since the present regimen was not associated with end organ-related toxicities even in patients with pre-existing hepatic and renal insufficiency, cytokine release from damaged tissue was minimized. Alternative conditioning regimens incorporating either antithymocyte globulin or anti-CD2 antibodies along with CSA have also been successful in attenuating aGVHD.[12,20,21] However, these regimens necessitated the use of subsequent DLI to establish full

donor chimerism with the increased risk of subsequent GVHD.[38–40,44]

The high response rate and OS in this high-risk population demonstrates that this RIT regimen was effective for disease control, even in patients with active disease at the time of the transplant. Surprisingly, neither age nor donor type (sibling or unrelated donor) had an impact on the incidence or severity of aGVHD or survival. As in other reports, survival was correlated only with time from diagnosis to the allogeneic HSCT and the number of prior therapies administered.[10–12,40] The prolonged course of MMF was not associated with an increased incidence of infection or disease relapse.

We acknowledge that our study reports on a limited number of patients with various diseases treated at a single institution. While the post transplantation follow-up is relatively short, we have not observed late graft failures or unexpected toxicities. The results of this novel approach to RIT allogeneic HSCT are encouraging. A larger multicenter trial is needed to confirm our results and assess the long-term benefit of this approach. Additional clinical and correlative studies are also needed to define the role of either ECP and/or pentostatin in prophylaxis for GVHD.

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